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"A Contribution to the Anatomy of Connective Tissue, Nerve, and Muscle, with special reference to their connexion with the Lymphatic System." By G. Thin, M.D.

I published in the 'Lancet' of the 14th February of this year a paper entitled "On the Lymphatic System of the Cornea," in which I endeavoured to show that the canals in that structure in which the nerves lie communicate with the lacunæ, that the straight canals and lacunæ are connected by means of a continuous layer of flat cells, the margins of which are indicated by the well-known action of nitrate of silver, and that these cells are not the anastomosing so-called cornea-corpuscles, but that the flat cells line the lacuna, while the branched cells fill the cavity.

I have lately undertaken a series of further investigations on the same subject.

In order to corroborate the results yielded me by the nitrate of silver, I availed myself of the well-known property which hæmatoxylin possesses of specially staining the nuclei of cells. I allow the cornea to remain in the solution until it is perfectly saturated. Subsequent maceration in acetic acid removes the hæmatoxylin from the fibrillary substance before it bleaches the nuclei. On comparing a cornea so treated with successful preparations of the cornea-corpuscles as obtained by chloride of gold, it is found that the number of cells demonstrated by the hæmatoxylin exceeds by several times that found in the gold preparation, affording direct proof of the existence of other cells in the cornea than those known as the cornea-corpuscles.

If a vertical section of the cornea is so treated by hæmatoxylin and acetic acid, in many of the clefts in the fibrillary substance, in which, as is well known, the cornea-corpuscles are situated, several nuclei are seen, proving in another way the existence of a greater number of cells than those hitherto accepted by anatomists.

But in addition to the proof afforded by staining the nuclei of the cells, I have, by the application of a new method, been able to isolate (and thus demonstrate beyond all further possibility of doubt their existence in the cornea) a large number of cellular elements, the varied size and shape of which distinguish them not only from the cornea-corpuscles, but from any anatomical structures that have been as yet described.

If a cornea is placed in a saturated solution of caustic potash, at a temperature between 105° and 115° Fahrenheit, it is reduced, in a few minutes, to a white granulated mass of about a fourth of its previous bulk. In a small piece of the diminished cornea, broken down with a needle and examined under the microscope in the same fluid, it is found that the only visible elements are a great number of cells. If the con-

junctival epithelium of the cornea has not been previously removed, the cells of that structure can be recognized amongst the others; and if the mass under examination has not been too much broken up in manipulating, groups of them may be seen in direct anatomical continuity with long narrow flat cells, which belong to the elements that have been for the first time brought to light by the potash solution.

But the cells of the anterior or surface-epithelium form a very small proportion of the number. The smallest piece that can be removed by the needle from a cornea which, before being put into the solution, has had this epithelium scraped off and Descemet's membrane removed, shows under the microscope a multitude of cells. Of the branched corpuscles, the fibrillary substance, and nerves, not a trace is visible.

The form of these cells is so various that it would be difficult to construct a series of types under which every individual cell could be brought. They seem in their development to have assumed any modification of form that is necessary to enable them to fit accurately the cavities and fibrillary bundles to which they are applied.

Those whose outlines do not permit their being accurately described as belonging to a strictly defined type, are many of them somewhat quadrangular or triangular in form, or club-shaped, with a short or long projecting process. Of fixed and definite types are long narrow rods, ending obliquely at the point, and oblong cells intersected at one end by a notch, which receives the extremities of two of the long cells that lie parallel to each other.

I do not attempt to give an exhaustive account of the various forms assumed by these cells. A better idea than can be given by any description will be got by an examination of figs. 1, 2, 3, Plate VIII., in which many of them are represented; but an examination of the first prepared cornea will show that there are many forms and modifications which have not been drawn.

The cells are granular in appearance, with sharp clear outlines. The terminal surfaces of the long cells can often be seen to be finely serrated; and so closely do they fit each other at these points, that sometimes a high magnifying-power is necessary to discover the suture-like line by which the junction is indicated.

The nuclei of all the cells have nearly the same length, but in the narrower cells the nucleus is often much compressed transversely.

The long cells are many of them 0·09 millim. long and from 0·006–0·003 millim. broad; the shorter cells are broader. Those 0·06 millim. long are generally about 0·009 millim. broad. A length of 036 millim., with a breadth of about 0·015 millim. is common; others are 0·03 millim. long and 0·012 millim. broad.

I have chiefly examined the cells in the cornea of the ox, sheep, and frog, and have found no important differences either in shape or arrangement.

In examining portions of the cornea which have been as little dis-

turbed as is consistent with the maintenance of transparency, groups of cells are found massed together *in situ*, as they have been left by the dissolving out of the fibrillary substance by the potash: these are found chiefly in two forms. Transverse masses of the anterior epithelium are found sutured to long narrow cells, which sometimes seem to join them at an angle.

Further, flat quadrangular masses of a single layer of cells are found formed in the following manner:—Of two opposite sides the external rows are formed of more or less rounded and angular cells, to which are joined long narrow cells that lie parallel to each other. Those from each side respectively meet in the centre, where they join. The remaining sides of the quadrangle are formed by a side view of these various cells, where they have been detached from the adjoining ones in the breaking down of the cornea mass.

The coincidence between the breadth of the long narrow cells and the fibrillary bundles of the cornea-substance, as seen when prepared by the ordinary methods, is evident, the continuous planes formed by their junction indicating that they form layers between which it is enclosed. According to this view, the ground-substance is everywhere encased in a sheath of cellular elements.

Bowman's corneal tubes I believe to include both the straight canals described in the paper above referred to and the spaces between the long cells widened by injection, chiefly the latter.

Although I have nothing to add to the description of the mode of preparation which I have already given, I must state that there are conditions of success, as to the nature of which I have not yet come to a definite conclusion. Sometimes the same solution, applied at the same temperature to different corneæ, succeeds in one and fails in another, and sometimes a solution prepared with every precaution has failed to afford me any result. The two essential conditions to success are complete saturation and temperature. I have never succeeded with a temperature above 120°, nor with one below 102°; and so sensitive is the solution to moisture, that preparations sealed in it with asphalt seldom keep longer than one or two days, except in very dry weather. On a damp day I have known a successful preparation left on the object-glass disappear in six hours. The corneal mass may be kept unaltered for at least some weeks in the solution by running sealing-wax round the stopper of the bottle.

A perfectly successful preparation shows nothing but the cells. Unsuccessful preparations, especially those prepared with too hot solution, show globular masses unlike any anatomical element; others, especially those prepared at too low a temperature or with imperfect saturation, show masses of hexagonal crystals like those of cystin.

To sum up, I believe that there exists in the cornea:—

I., the fibrillary ground-substance, which is pierced by straight canals and honeycombed with cavities;

II., flat cells, which everywhere cover the fibrillary bundles of the former and line the entire system of the latter;

III., the cornea-corpuscles of Toynbee and Virchow; and,

IV., the nerve-structures of the tissue.

The cornea-corpuscles and the nerves lie free in the canals and cavities, and between them and the epithelium there is a fluid-filled space which permits the passage of lymph-corpuscles.

It is therefore proper to regard the canals, cavities, and inter-fibrillary spaces as forming a continuous and integral part of the lymphatic system, the latter having to the former the same relation that blood-capillaries have to the veins.

The junction of the flat cells of the fibrillary substance with the epithelium of the surface justifies the inference that the intercellular spaces in the anterior epithelium of the cornea communicate with the lymph-spaces in the ground-substance, and that the position of nerve-fibrillæ between the epithelium is a continuation of the similar relation that has been demonstrated in the substance of the structure.

It is a reasonable hypothesis that what can be definitely established for the cornea holds good for the other forms of connective tissue.

I have accordingly submitted tendon to an examination by different methods, with the view of obtaining evidence of the existence in that structure of cells other than those arranged longitudinally between the bundles, the nature of which has lately been carefully investigated by Boll, Spina, Ranvier, and others.

If the tendo Achillis of a frog, or the tendons of a mouse's tail, fixed according to the ingenious method described by Ranvier in his first paper*, are treated by nitrate of silver, care being taken to avoid friction of any kind, it is found that every part of the free surface of the bundles is covered by a continuous epithelium. In the tendo Achillis of the frog I have seen lymphatic capillaries distributed over this surface; and the epithelial markings can be traced from the cells covering the bundles into those of the vessels. A preparation from the tail of the mouse, showing this epithelium, is represented in fig. 4, Plate IX.

If sections of tendon are placed for several hours in a strong solution of extract of logwood and alum, and the dye then washed out by concentrated acetic acid, it is found that while the fibrillary substance becomes clear and transparent, the nuclei retain their colour. This is best done under a cover-glass and under the microscope, as the effect of the acid, if kept too long in contact with the preparation, is to discolour the nuclei also; the weight of the covering-glass is sufficient to prevent the otherwise invariable distortion of the preparation by the acid. If the preparation is intended to be permanent, all traces of the acid must be removed by a current of distilled water.

The effect of this treatment is to show that there exists in tendon a

* Archives de Physiologie, 1869.

far greater number of cells than can be seen in the most successful gold preparations. The figures illustrating the structure of tendon usually given by investigators account for only a portion of the cells whose existence is thus proved—that portion, namely, which consists of the rows of cells occupying the stellate spaces, and which colour easily in gold and carmine. In longitudinal sections, prepared by the method I have above described, not only are the nuclei much crowded together, but two are frequently seen on the same level, and applied to the opposing surfaces of contiguous bundles. In transverse sections a similar arrangement is found. The nuclei between the bundles are very numerous; two are often found together on opposite bundles; and in one stellate space three and four nuclei can often be found at the same level.

This is clearly a condition to which the so-called division of the nucleus is not applicable.

If we believe that each of these nuclei represents a cell, the conclusion is inevitable that, in addition to the cells hitherto described and occupying the centre of the stellate spaces, there exists another and very numerous class of cells applied to the surface of the bundles.

This effect of hæmatoxylin and subsequent action of acetic acid on sections of tendon is perfectly analogous to that similarly produced in sections of cornea.

The treatment of tendon by the potash solution has seldom yielded me satisfactory results; but when it has succeeded, I have found confirmation of the inferences I draw from the effect of the saturated solution of hæmatoxylin. A reference to figure 7 (Plate IX.) shows that while many of the cells isolated by the potash correspond to those found on the surface, others are similar to the long narrow cells that cover the fasciculi of fibrillary tissue in the cornea, and do not resemble in shape, even approximately, the superficial elements defined by nitrate of silver. Although I have not succeeded, as in the case of the cornea, in reducing tendon to a mass of these cells, I consider it a fair inference that the long narrow cells I have seen are samples of cells that invest the fasciculi of fibrillary tissue.

The comparative difficulty in successfully treating tendon by potash is probably due to the denseness of its structure.

It is in regard to the branched cells, which I hold to be the analogues of the branched cornea-cells (corpuscles), that the important fact demonstrated by Spina, that it is on the surface of the cells that the fibres of elastic tissue are formed, specially applies.

In the centrum tendineum of the rabbit the continuity of the flat cells, which in silver preparations are considered to indicate lymphatic vessels, with cells covering the fibrillary substance can be shown to a greater or less extent, according to the success which has attended this difficult manipulation. That it often succeeds in patches, is shown by

the plates that illustrate works on this subject, although the influence of Von Recklinghausen's doctrine (namely, that wherever an epithelium is found a lymphatic capillary must be supposed to exist) has led to what I believe to be their true nature being overlooked.

From a similar cause to that encountered in tendon, the complete reduction of the dense corium of mammals by potash is very difficult; but by treating thin sections of fresh cutis, isolated flat cells are found.

In the cutis of the frog, the bundles of fibrillary tissue are arranged in parallel layers, and the corium being thin, the demonstration of flat cells is easier. And here the continuity of these cells with those of the rete Malpighi is evident, in the same way as the cells of the anterior epithelium of the cornea are continuous with the flat cells of the interior of the structure.

Figures 11 and 12, Plate X., represent flat cells from the skin of the ox and frog.

I make the same inference in regard to the communication of the spaces between the cells of the rete Malpighi with the lymph-spaces of the corium, that I make in regard to the similar arrangement in the cornea, both as regards anatomical continuity and in regard to the position of the nerves in the spaces. Langerhans has described the network of nerve-fibres in the rete Malpighi, and, in that of the cutis of the rabbit, the rich network in the spaces between the cells is not very difficult to demonstrate. In the skin of the finger, I have traced a medullated nerve as high as the third layer.

Ranvier, in that part of his essay which treats of the *éléments cellulaires du tissu conjonctif lâche*, describes an entirely different anatomical element from that on which the authorities with whom he is in controversy had fixed their attention. The cells figured by him* are the same as those isolated by potash when very thin pieces of skin or areolar tissue are operated on. As described by him, they are applied closely to the bundles. But when he attempts to show that the connective-tissue corpuscle of Virchow does not exist, and that the appearances by which it is distinguished depend on an optical delusion, I believe him to be mistaken. In skin and subcutaneous tissue the chloride of gold brings out in the clearest manner the existence of nucleated cells with long projecting processes stretching between and around the bundles, the whole of the cells being connected by the anastomoses of their processes. So complete is the analogy between skin and tendon, that it would be easy to find parts of a successful gold preparation of skin where the diagnosis between skin and tendon might be difficult.

Figures 13 and 14, Plate X., illustrate the appearances presented by the branched cells in skin.

A history of the opinions held regarding the structure of the connective tissues since the time of Schwann is equally beyond the scope of

* *L. c.* p. 483.

this paper and my acquaintance with the literature of the subject. It is, however, well known that while twenty years ago the so-called connective-tissue corpuscles were believed to be concerned in the formation of elastic tissue, with the development of Virchow's doctrine of cellular pathology, this opinion seems to have been gradually abandoned, even by those who, like Virchow himself, had originally maintained it. Ranvier, whose investigations seem to have been conducted in singular independence of contemporary theories, holds that the first step in the appearance of elastic tissue is the formation of "*granulations réfringentes*," traceable in the fully developed fibres.

In the spring of 1873, while investigating the structure of the touch-corpuscles of the finger, I found that the much-discussed cellular elements of these bodies, which colour in gold and carmine, anastomose with each other by means of fibres that resist prolonged maceration in concentrated acetic and dilute mineral acids, and I described them, in the account I gave of the results of my investigation, as "elastic tissue fibres." At the same time I found that similar cells and fibres form a thick network in the corium. Simultaneously, Spina made his exhaustive study of the connexion of the elastic fibres in tendon with the walls of the cell, to which I have already referred.

Since that time, I have continued to subject the skin and subcutaneous tissue to treatment by different methods, and the results have been confirmative of those I obtained in Vienna. Shortly expressed, the conclusion I have come to is, that, in skin, all the branched cells form elastic tissue on their surface and on their processes, and that there is no elastic tissue anywhere that is not so formed.

The cells found in connective tissue are divisible, as I believe, into two distinct classes. There are, first, the flat cells which never branch, and which, when treated by nitrate of silver, present appearances identical with those produced when the flat cells of serous membranes are similarly treated; secondly, there is the system of branched cells in its various forms. As contrasted with each other, they may be described simply as the flat and branched cells of connective tissue*. Between these two classes of cells there is no transition and no anatomical continuity. The forms of the branched cells embrace all the gradations between the fine network of a lymphatic gland and the anastomosing network of the strong fibres in skin and tendon. They are distinguished by their processes, their capacity to form a substance that resists acetic acid—the power, namely, of forming the resisting element specially characteristic of elastic tissue. That they do not all exercise this latter power to the same degree, does not constitute a sufficient difference to make it necessary to regard them as separable into classes essentially distinct.

The ligamentum nuchæ may be taken as the type of the stronger forms

* To flat cells the term *placoids* has been applied by Dr. Burdon Sanderson, the equivalent of the German *platten*.

of elastic tissue; and I select it for this reason to prove the cellular origin of its fibres.

If a thin piece of the ligamentum nuchæ is strongly coloured by chloride of gold and gently teased in glycerine, there will be found a number of oval nuclei lying loose amongst the fibres. But careful examination shows similar nuclei still adhering to many of the latter; and in some instances the remains of the protoplasm of the cell can be seen around the nucleus and adherent to the fibre. The nucleus and cell-remains are often found at the point of the division of a fibre into two, and indicate the original processes of the cell in the embryonic state.

If a portion of the same gold-stained ligament is further placed in a very strong solution of hæmatoxylin and alum for twelve hours, and then carefully spread out for examination, the appearances will be found to have considerably changed. If the preparation has not been roughly handled, the astringent effect of the latter solution has caused the clear outlines of the individual fibres to disappear, and, in their stead, there are flat bluish bands in which fine dark lines connecting oval swellings are seen. The latter are the nuclei, and the lines are permeable canals in the elastic fibres, which have become filled with the hæmatoxylin solution. Both these conditions are depicted in figures 15, 16, and 17, Plate X.

The formation of the elastic substance on the surface of the cell, as described by Spina in tendon, applies universally, and also holds good for the cell-processes. But the part of the cell-body that does not enter into the formation of this resisting substance, so far from sharing the strength of the new tissue, becomes more easily disintegrated than at an earlier period of its development, and can be found only when the tissue is cautiously manipulated. But sufficient staining with gold, and care in operating, will demonstrate the cellular origin of elastic fibres in whatever tissue they occur.

Virchow, as is well known, vindicates for his connective-tissue corpuscles the character of a connected chain of plasmatic canals, and I have remarked above regarding the tubular nature of the fibres of the ligamentum nuchæ. That every elastic fibre is permeable to fluid is highly probable, though not yet proven. This tubular nature of the larger fibres has produced one of the difficulties of the recognition of the connexion of the fibre with the cell. The chloride of gold colours the protoplasm of a cell, with which a fully developed fibre is continuous, a faint purple; and when the tinting is continued into the process, it is the contents of the tubular space that are coloured. The elastic fibre, unless carefully examined in a good light, is apt either to escape observation, or seems to run past the cell without being in continuity with it.

This difficulty has been increased by a chemical difference between the cell and the elastic tissue to which it gives origin, so that many reagents and modes of treatment, that by potash-lye for instance, dissolve the cell but leave the fibre untouched. Hence the methods that have been most

used for establishing the individual characters of elastic tissue have been instrumental in producing erroneous notions as to its origin.

Thus we have in skin, as in tendon, bundles of fibrillary tissue everywhere covered with flat cells, and, in the interstices of the bundles, the analogues of the branched cells of the cornea, producing a ramifying network of elastic tissue.

In gold preparations of the skin, the blood-vessels and nerves can be followed between the larger fasciculi, analogously to the position of the nerves in the cornea.

Fascia differs from skin and tendon only in so far as its flatness permits and necessitates a change of form in the flat cells, and the easy study of their arrangement and nature by nitrate of silver. If a half per cent. solution is injected under the skin of a mouse's back and the animal killed in from five to ten minutes afterwards, and the skin of the back dissected off, the fascia which has been in contact with the silver is recognized by its milky whiteness and œdematous condition. If spread out carefully on the object-glass in glycerine and exposed to sunlight, it is seen to be plated over with oblong or slightly rounded cells with large nuclei. Figure 8, Plate IX., is a sketch from a part of a preparation so obtained. The cells separated from the same structure by potash are represented in figure 9, Plate IX.; it will be observed that they are identical in appearance. Figure 10, Plate IX., illustrates the very large flat cells, with their nuclei, that cover the fascia of the muscles of the thigh of the frog.

Frequently, but not so constantly, the branched cells are also stained by the silver, and they are generally found at a different focus.

Ranvier ('Archives de Physiologie') has described flat cells on the sheaths of nerve-fasciculi and the investing membrane of nerve-bundles as constituting a lymphatic sheath.

By means of the saturated potash solution I have been able to satisfy myself that, not only are the nerve-bundles surrounded by lymphatic sheaths, but that each medullated nerve-fibre is invested with a layer of flat cells. This layer is closely applied to the medulla, and is internal to the sheath of Schwann. It is composed of extremely fine and delicate cells, and their demonstration by potash succeeds less frequently than does that of the cornea-cells; they are (as far as I have seen) without exception long and narrow, often tapering to an exceedingly fine point. In the finest forms their cellular nature is only to be distinctly made out by a magnifying-power of 700 or 800 diameters. Figure 18, Plate X., represents varieties of these cells and their relation to the medulla. Their length varies from 0·075 to 0·036 millim.; many of them are not more than 0·0015 millim. broad. Appearances are sometimes seen that would seem to indicate that the sheath of Schwann (tubular membrane) is lined by a layer of flat cells, distinct from that covering the medulla (white substance). The medulla, when treated by potash, presents

a series of bulgings, so that its lateral (optical) borders are designated by irregularly waving lines. One set of delicate cells can be seen closely following the sinuosities of the substance, while another set, more external, lie in a straight direction parallel to the longitudinal axis of the fibre. Where the medulla is constricted, there is a clear space between these two sets of cells, which are in contact at the convexities formed by the bulgings.

By adhering to the broad principle that wherever there is a nucleus there is a cell, the existence of a great number of cells surrounding the medulla of a nerve-fibre can be demonstrated in another way. If a nerve is placed in absolute alcohol for twenty-four hours and then very gently disentangled from the sheath in glycerine, a cover-glass put on and solution of hæmatoxylin drawn through the field by filter-paper, the nuclei of the fibres stain first, and their number soon becomes very striking. If the field is allowed to become saturated and obscure with the dye, and then subsequently cleared up by acetic acid, those fibres which have not suffered by the manipulation are literally dotted over with nuclei. The number is so great as at once to dispel the idea that they can be accounted for by the sheath of Schwann. The nuclei of the sheath can often be distinguished from the others by their more external position relative to the nerve and a deeper tint. Figure 19, Plate X., is drawn from a preparation made in this way.

The ring which, as Ranvier was the first to show, snares the medullated fibre is well seen when the nerve is treated by absolute alcohol or the saturated potash solution, both of which leave the medulla untouched. As at the seat of this constriction the medulla is deficient, and as the nerve-fibre is bathed in lymph, it is evident that there must be at these points a very intimate connexion between the lymph-fluid and the axis-cylinder of the nerve. This has been already indicated by Ranvier in his essay on the lymphatic nature of the nerve-sheaths, and receives greater force now that we know that flat cells, indicative of lymphatic structures, are situated on the fibres themselves.

The use of hæmatoxylin is as advantageous in demonstrating the large nuclei of the flat cells of the nerve-sheaths as it is in showing those of tendon.

Ranvier has observed that in transverse sections of nerves the sheaths and connective tissue surrounding the fibres stain more deeply with carmine than the surrounding tissue does. I have made a similar observation in the nerves of the skin in gold preparations which had been macerated in acetic acid. In this case the concentrically arranged connective tissue of the nerves is conspicuous by its pearly whiteness. But as we know that the surrounding corium is equally rich with the nerve in lymphatic structures, the cause of the difference in colour must be sought elsewhere, and will probably be found in relative differences in regard to the arrangement of the elastic tissue.

By combining several methods of investigation, I believe I have succeeded in clearing up some points in the anatomy of muscular fibre, by which it will be seen that, as regards the lymphatic system, muscle occupies a position almost identical with tendon and connective tissue generally.

If fresh muscle is deeply stained with hæmatoxylin and then treated by acetic acid and gently teased, the perimysium of the bundles and its very fine continuation around each fibre are seen to be studded with large round nuclei, which are far more numerous than those of the branched cells, which are also seen. The round nuclei belong to the flat cells of the perimysium.

I have been able to demonstrate the character of the cells by teasing the living muscle of the frog in aqueous humour, and thence transferring the separated fibres to the nitrate-of-silver solution. The usual sinuous lines are then seen both on the general and special perimysium. This is represented in figures 5 and 6, Plate IX.

When muscle is treated by the saturated solution of potash, as above described, the fibres are found unaltered, the striated appearance being well marked. There is no vestige left of the perimysium. On the naked surface of the sarcolemma, a number of round distinct nuclei are seen; and when they happen to be on the edge of the fractured fibre, it is seen that they are situated on its outer surface.

If the saturated potash solution in which the muscle is placed is kept for about an hour at a temperature of 110° Fahrenheit, and then allowed to cool gradually, we find a further effect has been produced.

On breaking down a piece of the muscle into its individual fibres, we find that although some of these are unaltered, others have lost all their nuclei, and present the appearance of a coarse granular cylinder. But there is sometimes a transition stage seen of peculiar interest. On the surface of the fibre the outlines of a series of quadrangular cells make themselves visible, each cell having a distinct nucleus; and it is easy to satisfy one's self that the nuclei of the cells are identical with the nuclei seen previously distributed over the surface of the sarcolemma. These cells are sometimes also seen free in the solution, in which case they are generally more or less broken up, but sometimes they are seen isolated in perfect condition. Figure 21, Plate XI., shows the cells becoming demarcated on the fibre, and figure 22, Plate XI., their appearance when isolated entire. The sarcolemma is sometimes seen freed both from the cells and their contents; and in this case the striped cylinder which may be seen near it is beset with small perforations.

The sarcolemma of muscle is thus covered with flat cells, regular in appearance and outline, which resist the action of a saturated solution of potash.

But the action of the potash teaches us something more. A fibre is sometimes found apparently unaltered, smooth in its contour, and still showing something of the striated appearance, but showing no nuclei.

One or more round holes are, however, seen on the pieces of broken muscle-cylinder, the more conspicuous because the nuclei are absent; they are about the size of the blood-corpuscle of the frog. By changing the focus, it is seen that each hole is only on one side of the fibre. The sharp clearness of their outline shows they are not artefacts, but spaces in which the sarcolemma is wanting (figure 23, Plate XI.). A further action of potash is seen when a muscular fibre is found channelled with one or more canals parallel to the long axis of the fibre. The canals thus seen are uniform in breadth; they are formed by rows of vacuoles, which correspond in shape and size to the nuclei of cells. (I had observed in studying the cornea that the first stage of the destruction of the flat cells, in the potash solution, is a vacuole taking the place of the nucleus.) By changing the focus, it is seen that these channels are in the substance of the fibre. Smaller channels and single vacuoles are seen in different planes.

A more extended degree of the action of potash on a fibre is when the central canal has no longer sharp outlines and is seen to contain granular débris.

Treatment of muscular fibre by hæmatoxylin gives, as regards nuclei, results confirmatory of those got by potash, in so far as a still greater number of nuclei are seen internal to the sarcolemma than is indicated even by that method. To obtain the best results from hæmatoxylin, the fibres should be isolated before being dyed. The excess of colour being removed by acetic acid, the nuclei become distinct; they are seen to be arranged in long rows, those of one row being in the same plane. Isolated nuclei are seen in different planes. An idea of their number is best formed from the appearance presented by the broken end of a fibre when it is turned upwards, giving a view equivalent to a transverse section. The whole thickness of the fibre is then seen to contain nuclei, in the arrangement of which something of a concentric disposition can generally be observed. The nuclei are large and oval, and contain one or two distinct nucleoli. If the substance of the fibre has been teased, it is seen that the fibrillæ are arranged in bundles which have an equal thickness, and isolated nuclei are seen adhering to their surface.

The inferences that are irresistible from these appearances prepare the way to readily understanding the more decided effects of an appropriate treatment by chloride of gold. The conditions of a successful examination of a muscular fibre by gold include the detachment of the perimysium from the fibre without injuring the latter, the obtaining good transverse views in the preparation, and the requisite degree of colouring. As it is impossible to ensure beforehand a combination of these favourable conditions, it is evident that, with equal care, success is not uniform. The results which I now give were obtained by teasing the muscles of the thigh of the frog in aqueous humour before staining with gold.

In the most perfect preparations thus obtained, the structure of a muscular fibre is seen to be almost identical with that of a fasciculus of

tendon. Longitudinally the fibre is seen to consist of parallel bundles of uniform width, separated by spaces that are indicated by distinct lines; and, distributed at intervals in the lines, are oblong nuclei, the long axis of which is parallel to that of the fibre. The breadth of the bundles is about the same as that of a secondary bundle in tendon; their surface is smooth and homogeneous (figure 25, Plate XI.).

A transverse view, corresponding to that of a cross section of tendon, shows the muscular substance intersected by stellate spaces, in some of which nuclei are seen, and, branching out from the spaces, a rich anastomosing network of fine dark lines divides the substance of the fibre into a number of compartments. Between the appearance I have just described and that of a cross section of tendon similarly prepared, the only difference is that, in muscle, the fields enclosed by the dark lines are dotted over by minute points, which may indicate the fibrillæ.

Nuclei are always seen in fibres successfully stained with gold, and especially when the fibre is separated by teasing into the bundles of fibrillæ; but their number is much less than that seen when hæmatoxylin is used. We have seen how, in the cornea, gold when it has deeply stained the nucleus of the branched cells leaves that of the flat cell invisible, while hæmatoxylin colours them both. So it is generally in the capillaries of blood-vessels. I have found that, in the capillaries of the muscles of the frog, these invariably consist of two layers—an internal epithelial layer, the outlines of whose cells are defined by nitrate of silver, and an external layer, into which a fine system of branched cells enters. Hæmatoxylin brings out the nuclei of the cells of both layers. The deep staining with gold, while it differentiates the layers by staining the internal (epithelial) more intensely than it does the outer (adventitious) layer, shows no nuclei in the epithelium, while the nuclei in the outer layer are well marked.

In applying to muscular fibre the experience thus acquired, we are warranted in concluding that the nuclei coloured in gold are those of cells that belong to the branched system, and which are the characteristic nuclei seen in the transverse view of a gold-stained muscle, while the great majority of those stained by hæmatoxylin belong to the flat cells of the lymphatic system.

The isolation of these cells is surrounded by difficulties, which are, however, surmountable. In fibres deeply stained by gold I have isolated long thin flat cells, lying amongst the fibrillæ, which are identical in shape with similar cells in the cornea. They were coloured uniformly deep purple, and a distinct nucleus was not visible. They are represented in figure 27, Plate XI.

Immediately investing the bundles composing a muscular fibre is the sarcolemma, which is externally, as I have shown, covered with flat cells. The property possessed by this membrane of resisting acetic acid is the cause of a characteristic appearance presented by a muscular fibre under

its influence. From the broken end a large uneven mass protrudes with thick everted lips, bending back over the membrane which forms a strangulating band round the neck of the protrusion. When this sheath is ruptured at different parts, the gelatinous substance, which forms a large proportion of the contents of the fibre, bulges out in masses as it swells. The fibrillæ, which do not swell under the acid, and which are imbedded in this mass, can be often seen, in teased gold or hæmatoxylin preparations, lying unaltered at one part of the field, while displaced masses of gelatinous substance are seen at another. (It is the disposition of this gelatinous substance in parallel bundles which is the cause of the peculiar effect of chloride of gold, represented in figure 25, Plate XI.)

The astringent effect of chloride of gold on the sarcolemma produces a very characteristic appearance. In manipulating a fibre, as a preliminary to its being hardened by gold, it sometimes happens that the membrane and the layer of muscle-substance adhering to it is rent longitudinally from the surface to the centre. In the gold solution it loses its cylindrical form, and spreads itself out as a broad band. This perfectly flat band is marked longitudinally with parallel lines, which are straight and equidistant from each other. The prolonged action of acetic acid does not alter the appearance of these lines or their mutual relations, but it makes visible a not very thick layer of gelatinous substance, which protrudes from under the edges of the band.

Without comparing this peculiar appearance in its most exquisite forms with the transition stages sometimes seen, in which one end of a fibre still retains its cylindrical form while the other end is flattened out, the observer might certainly doubt that he was looking at a muscular fibre. Interstices between the lines, and, in them, occasional oblong nuclei are sometimes visible.

The longitudinal lines are the optical expression of the septa between the bundles, which are seen through the transparent sheath; and that the fibres in these septa are formed by elastic tissue, is shown by their persistence when treated by acetic acid.

They differ in no respect from the septa and their contained nuclei, which are seen in muscular fibres that have retained their cylindrical form when the chloride of gold has produced that appearance.

Another occasional effect of the astringent action of gold is an exaggeration of the dimensions of the central canal. The upturned end of a fibre is sometimes seen in which there is the appearance of a wide central cavity, around which the contents of the sarcolemma form a thick rim. The mechanism of this appearance is explicable by the assumption that the sarcolemma becomes sufficiently unyielding to form an immovable surface, towards which the more yielding substance is drawn as the shrinking caused by the gold proceeds.

The sarcolemma is probably in very intimate connexion with the elastic network, the more superficial cells of which, with their prolongations, are

situated directly under and apparently in contact with it; and the numerous foramina seen in the cylindrical rod left by the potash solution, when the membrane has been loosened from it, are probably the points where the elastic fibres penetrate.

A muscular fibre is thus composed of a number of bundles resembling those of tendon, arranged parallel to each other, each bundle giving shelter to a number of fibrillæ, and separated from the neighbouring bundles by a space lined with flat cells. In the larger spaces lie branched cells, and in the smaller the projecting processes of the elastic fibres given out by the latter.

The large holes I have described in the elastic sheath afford passage to the nerves. When these have been traced, it is reasonable to infer that, here as well as elsewhere, they will be found to follow the lymph-channels. These holes not only permit the passage of nerves, but allow free communication between the lymphatic spaces within the fibre and those between the perimysium and the sarcolemma.

The abundance of gelatinous substance in a muscular fibre accounts for the phenomenon known as transverse cleavage, which is produced by the action of very diluted hydrochloric acid. I regard it as essentially equivalent to the effect produced in tendon when by similar treatment a bundle divides transversely into the flat plates known as the "Donderische Bänder," after the distinguished histologist who first described them.

To sum up these views regarding the structure of muscle in a few words, it might be said that a muscular fibre is a fasciculus of tendon in the bundles of which the primitive fibrillæ are imbedded longitudinally.

The small spaces at the points of junction of flat cells which colour deeply in silver, and to which allusion has been made by histologists, are seen in all tissues. They are always present when the colouring has been intense, and should, I believe, be regarded as playing an important part in the mechanism of the lymphatic system. They are especially well defined in the rete Malpighi of the frog, where it would be impossible to regard them as artefacts.

It is evident from the various anatomical facts above detailed, that the tissues may be said to be in an almost unbroken continuity with the lymph-system*. When a blood-corpuscle escapes from a capillary it is into the cell-lined spaces of this system that it directly passes, and there is manifestly no obstacle to the passage of the contents of the lymph-channel into the blood other than that formed by the wall of the capillaries, which alone separates the fluids of the lymphatic and vascular systems. We know that white blood-corpuscles can make their way

* In this connexion I quote from Ranvier's essay (*l. c.* p. 485) the following sentence:—"L'existence, dans le tissu cellulaire sous-cutané, de ces cellules plates, disposées à la surface des faisceaux, ne nous suggère-t-elle pas l'idée de voir dans le tissu conjonctif un vaste espace cloisonné, analogue aux cavités séreuses?"

through at the points of junction of the angles of the capillary cells*, and it is reasonable to suppose that these points are always permeable to fluids.

We have seen that there is a rich supply of lymphatic channels in the interior of a muscular fibre, and that the axis-cylinder of a nerve is probably in free communication with the lymph. The term "invagination," as applied to the relation of the nerves and blood-vessels of particular organs to the lymphatics, has no special physiological meaning, as it only implies that at certain parts a condition that is universal can, by special modes of procedure, be made capable of more easy demonstration. Every nerve-fibre and every blood-vessel is invaginated in lymphatics.

That there is a plasmatic circulation infinitely more comprehensive than that expounded by Virchow, is, as has been already remarked by Ranvier, a fact which anatomy has placed on an incontrovertible basis.

EXPLANATION OF THE PLATES.

The Drawings were executed by Mr. J. C. Ewart from my preparations. The objectives and ocular glasses referred to as indicating the magnifying-powers are those of Hartnack, with the exception of the No. XII. immersion-lens used in a few instances, which was made by Vériek, and has the power assigned to that number in his scale. Thus 3. VIII. means eyepiece No. 3 and objective No. VIII.

Plate VIII.

Fig. 1. Cells from the cornea of a frog which was treated by the saturated solution of potash. 3. VIII. Tube out.

Fig. 2. Cells from the cornea of the ox treated by solution of potash. 3. VIII. Tube out.

Fig. 3. Cells from the cornea of the sheep treated by solution of potash. 3. VIII. Tube out.

Plate IX.

Fig. 4. Tendon from a mouse's tail coloured by nitrate of silver. 3. VII. Tube out.

Fig. 5. Perimysium of muscle of frog. Silver preparation. 3. VII. Tube out.

Fig. 6. Perimysium of a muscular fibre of frog. Silver preparation. 3. VII. Tube out.

Fig. 7. Cells from tendo Achillis of frog by solution of potash. 3. VIII. Tube out.

Fig. 8. Fascia from dorsal muscles of the mouse. Nitrate-of-silver preparation. 3. VIII. Tube out.

Fig. 9. Cells isolated from the fascia of the dorsal muscles of the mouse by solution of potash. 3. VIII. Tube out.

Fig. 10. Continuous layer of flat cells investing the fascia of the muscles of the thigh of the frog. Nitrate-of-silver preparation. 3. VIII. Tube out.

Plate X.

Fig. 11. Cells of the cutis of the frog isolated by solution of potash. 3. VIII. Tube out.

Fig. 12. Cells isolated from the skin of the ox by solution of potash. 3. VIII. Tube out.

Fig. 13. The anastomosis of the cells by means of the elastic fibres. Gold preparation from finger, macerated in acetic acid. 3. XII.

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* Endothelium en Emigratie door Dr. Laidlaw Purves. Utrecht, 1873.

Fig. 14. Elastic fibres with cells. Section from gold preparation of skin of adult rabbit treated by concentrated acetic acid. 3. VIII. Tube out.

Fig. 15. Fibres from the ligamentum nuchæ of a three-days-old foal. Gold preparation. The nuclei and remains of the protoplasm of the cell stained. 3. VIII. Tube out.

Fig. 16. Ligamentum nuchæ of three-days-old foal stained in gold and hæmatoxylin. The central canal of the fibres indicated by the hæmatoxylin. 3. VIII. Tube out.

Fig. 17. Fibre from the same preparation as fig. 15. 1. XII. Tube out.

Fig. 18. Cells from the fibres of the sciatic nerve of the frog. Isolated by the saturated solution of potash. 3. VII. Tube out.

Fig. 19. Nerve-fibre from the sciatic nerve of the mouse. Treated by absolute alcohol, dyed with hæmatoxylin, and the excess of colour removed by acetic acid. 3. VIII. Tube out.

PLATE XI.

Fig. 20. Perimysium of a muscular fibre of frog stained in hæmatoxylin. Flat cells seen. 3. VII. Tube out.

Fig. 21. Muscle of mouse subjected to prolonged action of warm potash solution. The cells on the sarcolemma indicated. 3. VIII. Tube out.

Fig. 22. Flat cells from the sarcolemma of muscular fibre of ox. Isolated by prolonged action of warm potash solution. 3. VIII. Tube out.

Fig. 23. Muscular fibre of mouse treated by solution of potash. The holes in the sarcolemma seen. 3. VIII. Tube out.

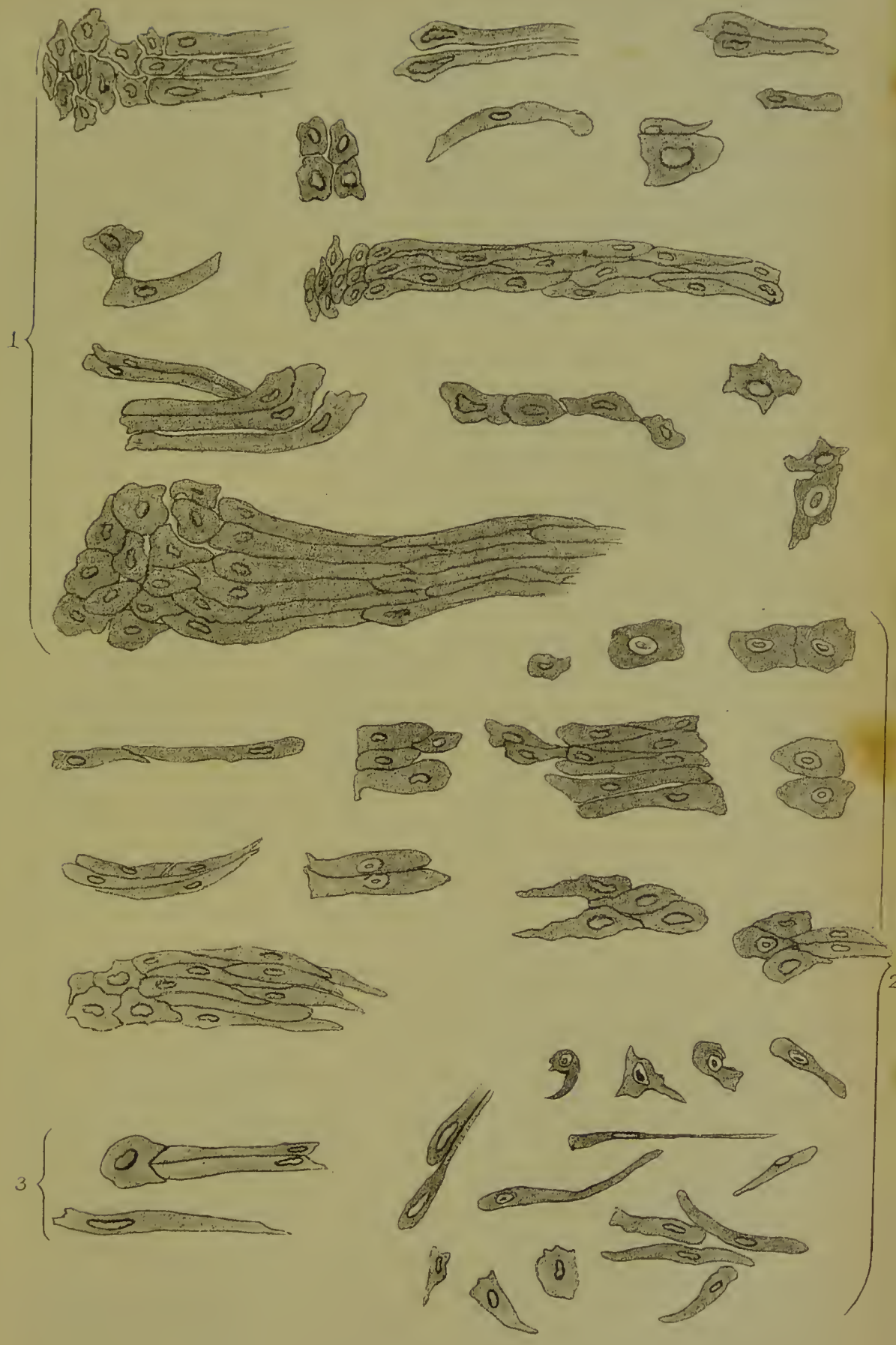
Fig. 24. Muscular fibre of frog treated by solution of potash. Canals indicated by nuclear vacuoles. 3. VIII. Tube out.

Fig. 25. Muscular fibre of frog. Gold preparation. Sarcolemma rent longitudinally and flattened. Septa dividing muscular substance visible. 3. VII. Tube out.

Fig. 26. End view of muscular fibre of frog. Gold preparation. Stellate spaces with nuclei and processes of branched cells, having the signification of elastic fibres, between the bundles. 3. VII. Tube out.

Fig. 27. Muscular fibre of frog. Gold preparation. The fibrillæ separated by teasing into bundles, between which long narrow flat cells are seen. 3. VII. Tube out.

Fig. 28. Muscular fibre of frog. Gold preparation. The central cavity seen much enlarged by the astringent action of the gold. 3. VII. Tube out.



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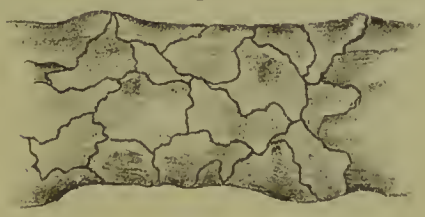

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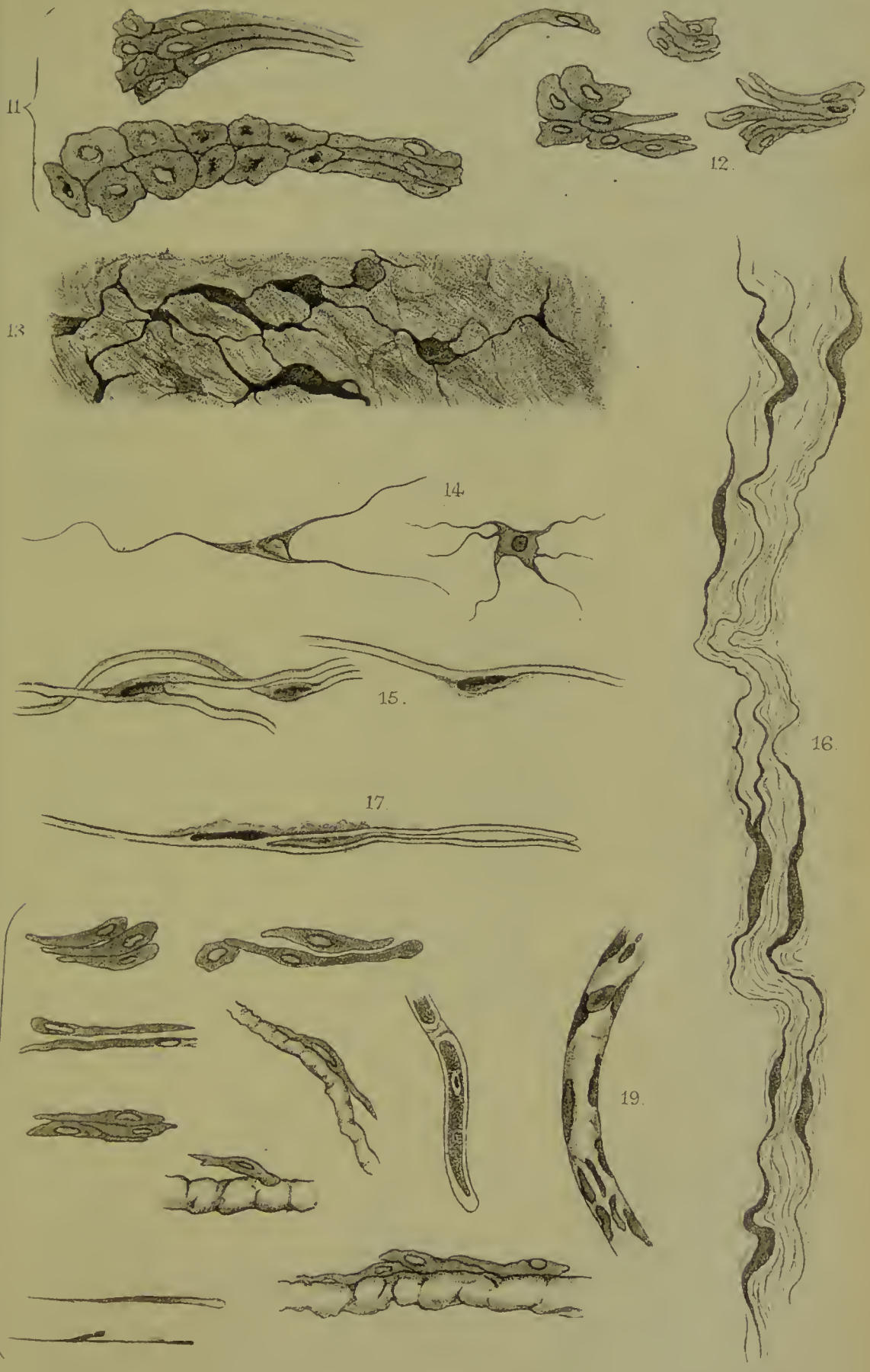

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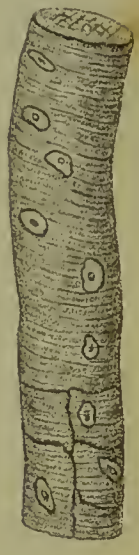

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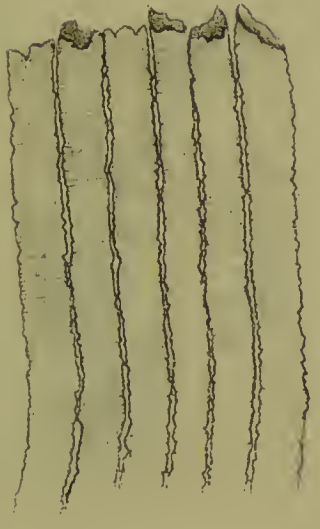

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